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13. ABSTRACT (Maximum 200 Words)

The overall goal of the project is to explore the idea that protein elongation factor EEF1A2 has an important causal role in breast tumour development and that EEF1A2 gene amplification and protein overexpression can be used as a breast cancer prognostic factor. In addition, we proposed to test the idea that EEF1A2 expression modulates sensitivity to cisplatin and taxol and that EEF1A2-inactivation could be used as a treatment for breast cancer.

In the one-year period since funding has been received, we have made progress in the following areas: 1. We have determined that approximately 25% of primary human breast tumours highly express EEF1A2; 2. We identified two human breast cancer cell lines that highly express EEF1A2 (MCF7 and BT474) and two that do not (MCF10A and BT549); 3. We created MCF10A and BT549 variants that highly express EEF1A2 and determined that EEF1A2 enhances the in vitro growth of theses variants compared to control; 4. We have created an EEF1A2 transgenic mouse; 5. We have created antisense oligonucleotides (AS) and siRNA that specifically inactivate EEF1A2; 6. We determined that the AS and siRNA inhibit the growth of MCF7 in vitro.

14. SUBJECT TERMS
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Table of Contents

Cover.....1

SF 298.....2

Table of Contents.....3

Introduction.....4

Body.....4

Key Research Accomplishments.....5

Reportable Outcomes.....5

Conclusions.....5

References.....5

Appendices.....6

Introduction

The development of cancer is associated with DNA amplifications that are believed to have a causative role in oncogenesis. We have previously found that amplifications of the EEF1A2 gene and its overexpression is associated with approximately 1/3 of primary ovarian cancers (Anand, Murthy et al. 2002). We found that EEF1A2 has the growth-enhancing and transforming properties of an oncogene (Anand, Murthy et al. 2002), consistent with it having a causal role in neoplasia (Thornton, Anand et al. 2003). The overall goal of the project is to explore the idea that protein elongation factor EEF1A2 has an important causal role in breast tumour development and that EEF1A2 gene amplification and protein overexpression can be used as a breast cancer prognostic factor. In addition, we proposed to test the idea EEF1A2-inactivation could be used as a treatment for breast cancer.

Body

Progress has been made in the following areas:

Task 1. Determine whether EEF1A2 enhances cell growth and tumorigenicity. To this end we have analyzed EEF1A2 expression in a panel of breast cancer cell lines (fig 1) and identified breast cell lines that highly express EEF1A2 (MCF7 and BT474) and those that do not do so (MCF10A and BT549). We have made a variants of MCF10A and BT549 that ectopically expresses EEF1A2 (fig 2-3). We have found that EEF1A2 expression in MCF10A and BT549 increases their in vitro growth rate (fig 4), consistent with the idea that EEF1A2 is a breast cancer oncogene. We are now testing the effect that EEF1A2 expression has on growth in soft agar and tumorigenicity in nude mice.

Task 2. Determine whether mammary-specific EEF1A2 expression enhances mammary tumorigenesis. To this end, we have derived an expression vector where the MMTV promoter controls EEF1A2 expression. This plasmid was used for three rounds of microinjection. Pups were weaned June 14, 2004 and we have identified 9 founder lines. We have 90 pups from an F1 cross utilizing 6 founder animals. We are currently testing each line for mammary-specific expression.

Task 3 .Determine the prognostic significance of EEF1A2 in breast cancer. There are two overall aims of this section a) Correlate EEF1A2 gene amplification with clinical and histological parameters, b) Create and EEF1A2 antibody and correlate EEF1A2 protein expression with clinical and histological parameters. As shown in Fig 5, increased EEF1A2 copy number correlates with reduced significantly reduced 10-year survival data in a n=142 cohort of breast cancer patients. We are now testing a larger (n=600) cohort. The generation of an EEF1A2 antibody for Task 3b has been more difficult. Our first attempt at immunization using the SHTTLLEAVDCIL peptide led to the generation of polyclonal rabbit sera that was equally reactive against EEF1A2 and the related eEF1A1 protein. This serum could therefore not be used for the necessary study. We have immunized rabbits with a new peptide and we are now testing this antibody.

Task 4. Determine whether EEF1A2 inactivation can be used as a treatment for breast cancer. To this end, we have identified an EEF1A2 phospho-orthioated antisense that can reduce EEF1A2 expression in the MCF7 breast cancer cell line (fig 6). This antisense appears to inhibit MCF7 growth (fig 7). We have also designed EEF1A2-inactivating antisense (fig 8) and these inhibit MCF7 growth in vitro (fig 9). We are currently testing theses agents for in vivo efficacy.

Key Research Accomplishments

We have determined that EEF1A2 gene amplification correlates with poor 10-year survival (n=142).

We have determined that approximately 25% of primary human breast tumours highly express EEF1A2.

We identified two human breast cancer cell lines that highly express EEF1A2 (MCF7 and BT474) and two that do not (MCF10A and BT549).

We created MCF10A and BT549 variants that highly express EEF1A2 and determined that EEF1A2 enhances the in vitro growth of theses variants compared to control.

We have created an EEF1A2 transgenic mouse.

We have created antisense oligonucleotides (AS) and siRNA that specifically inactivate EEF1A2.

We determined that the AS and siRNA inhibit the growth of MCF7 in vitro.

Reportable Outcomes

We created MCF10A and BT549 variants that highly express EEF1A2 and determined that EEF1A2 enhances the in vitro growth of theses variants compared to control.

We have made an EEF1A2/MMTV promoter plasmid

We have created an EEF1A2 transgenic mouse.

We have created antisense oligonucleotides (AS) and siRNA that specifically inactivate EEF1A2.

Conclusions

We have determined that EEF1A2 expression enhances the *in vitro* growth of breast cancer cell lines and that EEF1A2 gene amplification correlates with poor clinical outcome in women with breast cancer. These observations are consistent with the idea that EEF1A2 is a novel and important breast cancer oncogene. We have made an EEF1A2 transgenic mouse that may be an important animal model for breast cancer. Furthermore, we have identified EEF1A2-inactivating agents that may be used as anti-breast cancer treatments.

References

Anand, N., S. Murthy, et al. (2002). "Protein elongation factor EEF1A2 is a putative oncogene in ovarian cancer." Nat Genet **31**(3): 301-5.

Thornton, S., N. Anand, et al. (2003). "Not just for housekeeping: protein initiation and elongation factors in cell growth and tumorigenesis." J Mol Med **81**(9): 536-48.

Appendix

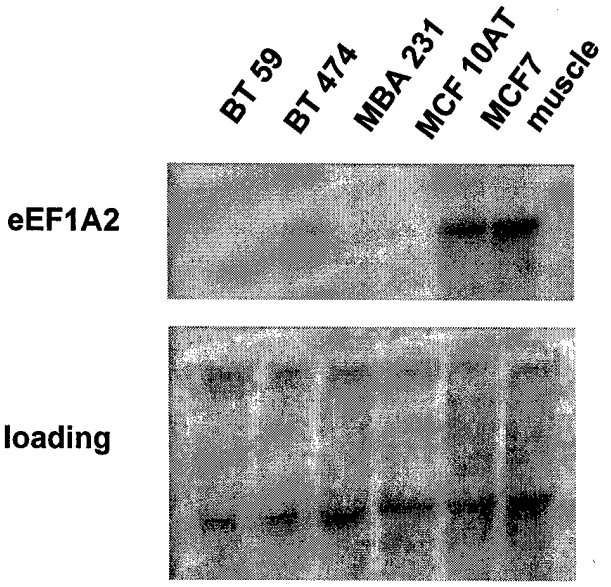

Figure 1. EEF1A2 mRNA expression in breast cancer cell lines. The breast lines BT 474 and MCF7 express detectable EEF1A2 mRNA while BT 59, MBA 231 and MCF10AT do not.

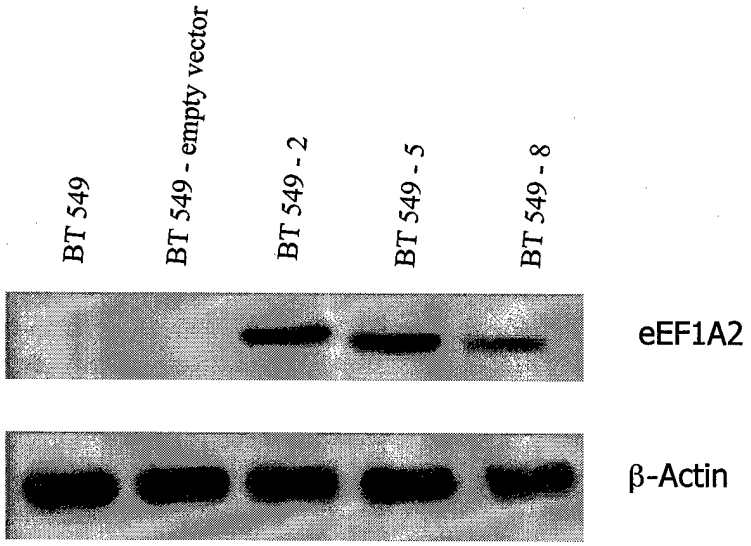

Figure 2. Creation of BT-549 cells ectopically expressing EEF1A2. The EEF1A2 non-expressing BT549 cell line was stably transfected with EEF1A2 and expression detected by Western blotting.

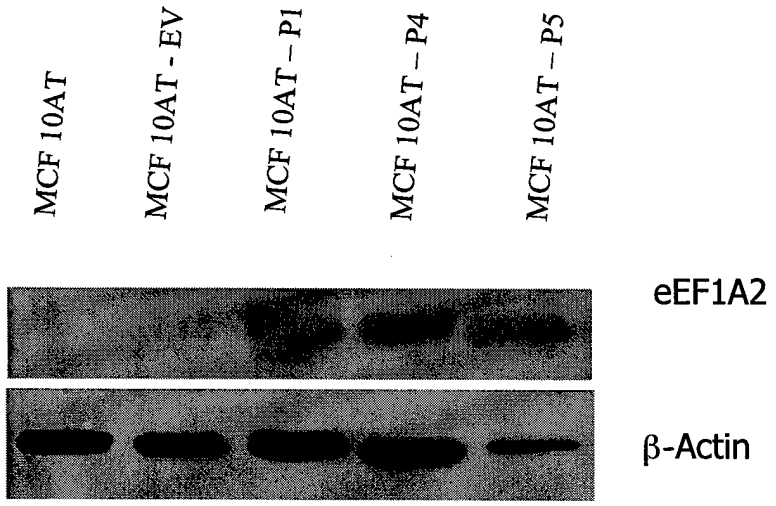

Figure 3. Creation of MCF-10AT cells ectopically expressing EEF1A2. The EEF1A2 non-expressing MCF10AT cell line was stably transfected with EEF1A2 and expression detected b y Western blotting.

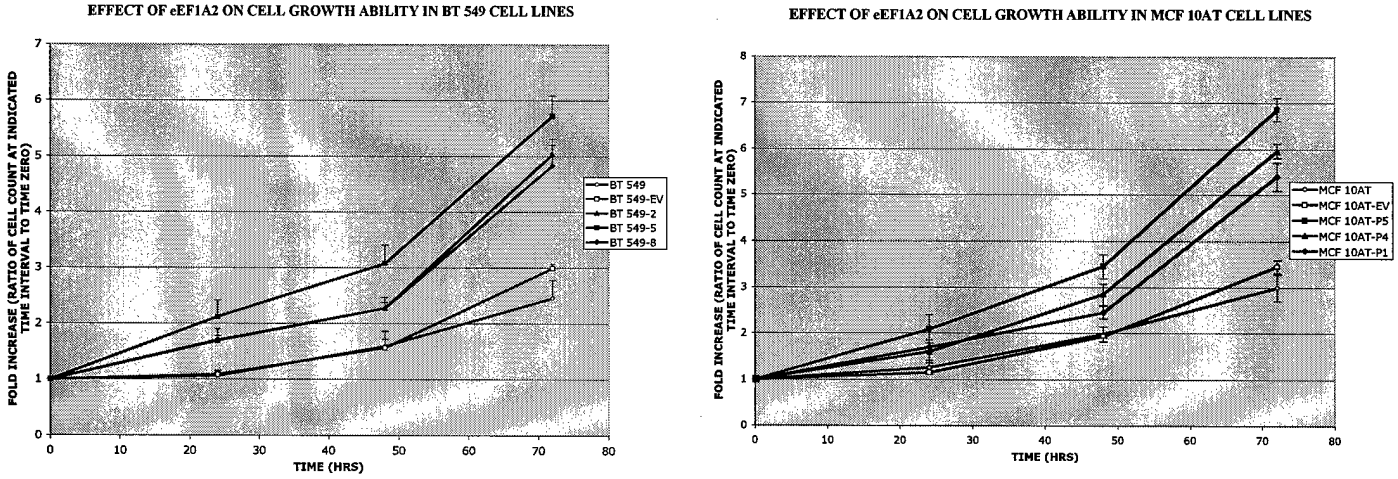

Figure 4. EEF1A2 expression in BT549 and MCF10AT enhances in vitro growth rate. The growth rate of EEF1A2-expressing variants of BT549 (left panel) and MCF10AT (right panel) grow faster than controls. Growth was measured by counting viable cells (Trypan Blue) at the indicated times following initial plating. Data points are the mean and standard deviation of triplicate measurements.

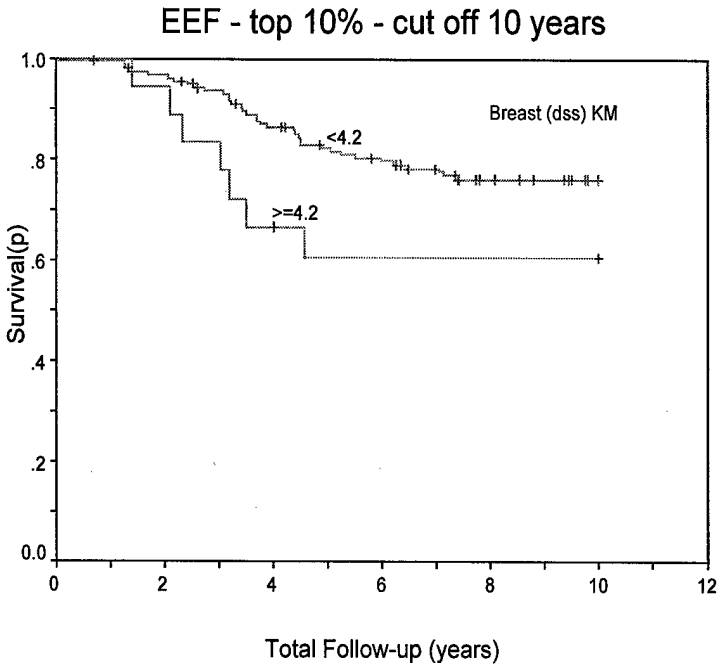

Figure 5. EEF1A2 increase in copy number is associated with reduced survival in breast cancer. Women with >4 copies of *EEF1A2* in their tumours (purple line) have a reduced probability of 10-year survival compared to those with normal *EEF1A2* copy number (green line)

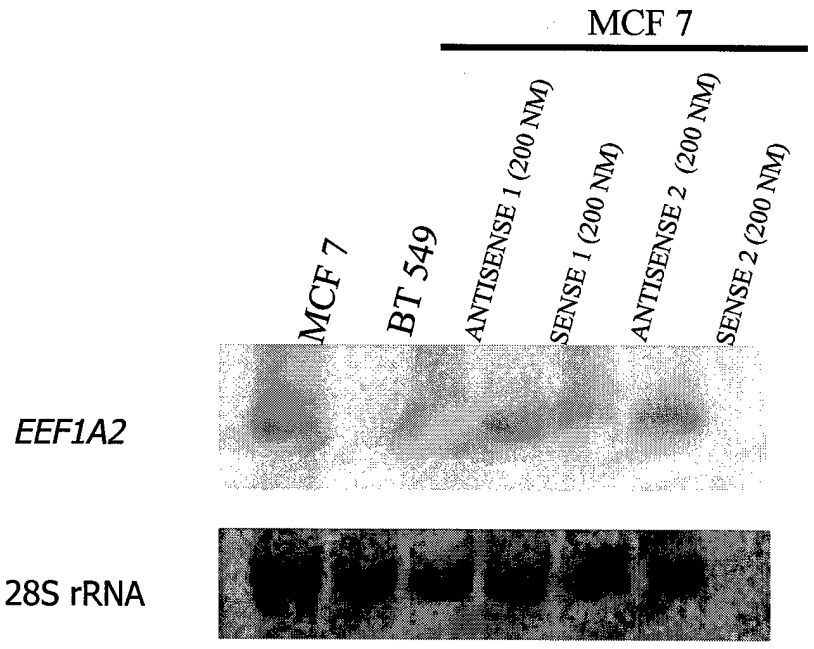

Figure 6. Antisense inhibition of EEF1A2. A) EEF1A2 antisense but not scrambled control reduced EEF1A2 levels in MCF7 cells.

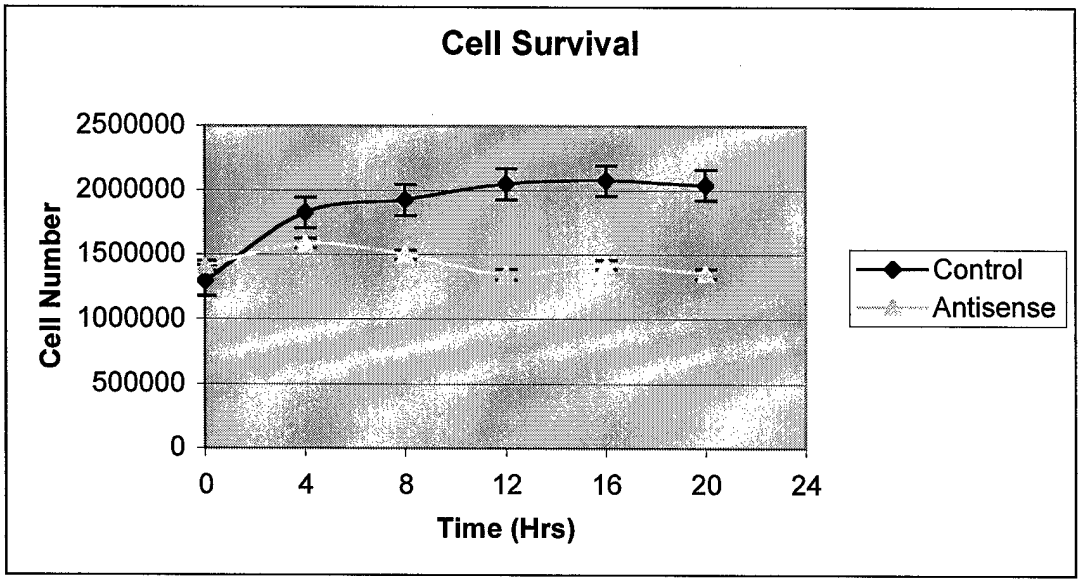

Figure 7. Antisense inhibition of EEF1A2 inhibits MCF7 growth. Antisense inhibits growth of MCF7 cells as measured by trypan blue exclusion. Data points are the mean and standard deviation of triplicate measurements. Antisense (Antisense 1 from fig 6) was used in a single dose of 200nM.

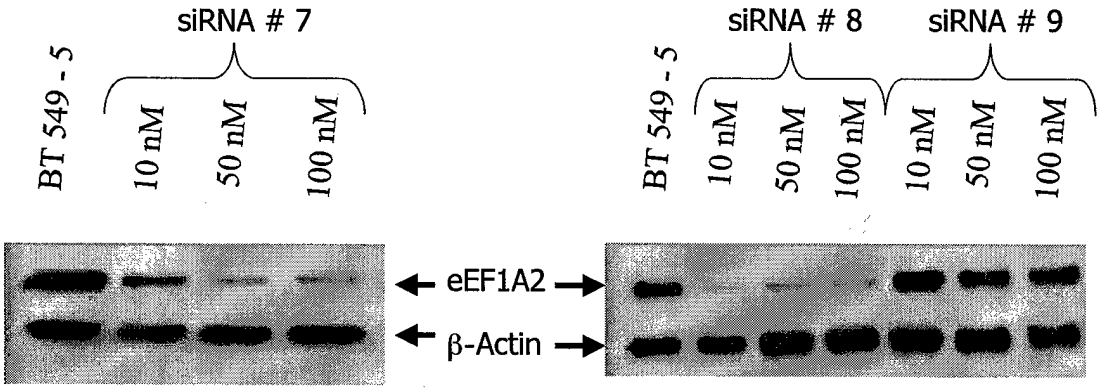

Figure 8. siRNA that inhibit EEF1A2 production. Three siRNA (Ambion) were tested for their ability to prevent EEF1A2 production. SiRNA #7 and #8 are effective.

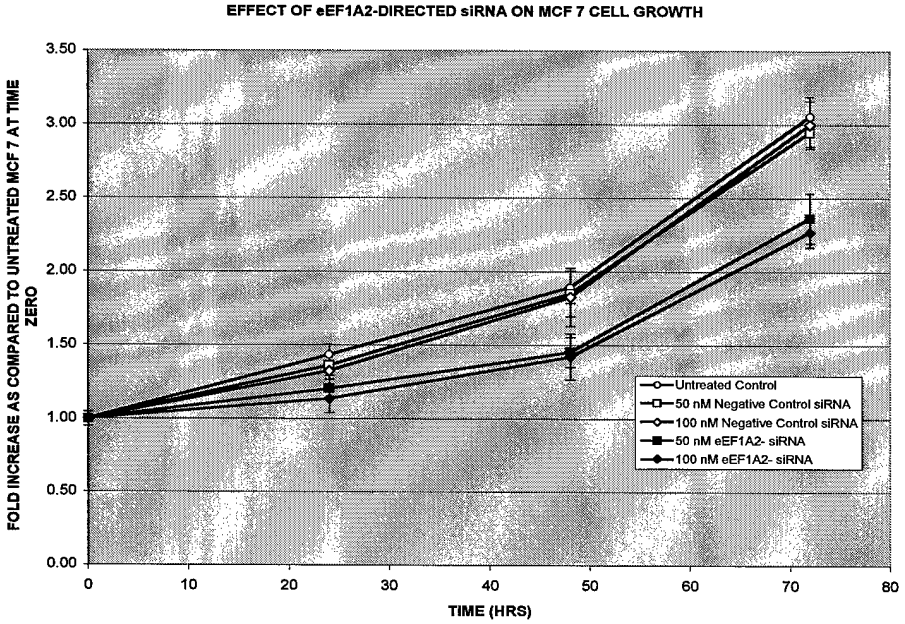

Figure 9. EEF1A2 siRNA inhibits MCF7 growth. siRNA (#7 from fig 8) inhibits in vitro growth of MCF7 cells as measured by trypan blue exclusion. Data points are the mean and standard deviation of triplicate measurements.